

HOMOCYSTEINE AND COGNITIVE IMPAIRMENT IN PARKINSON'S  
DISEASE: A BIOCHEMICAL, NEUROIMAGING AND GENETIC STUDY

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## ABSTRACT

The role of the plasma level of homocysteine (Hcy), as a primary outcome, and the effect of silent cerebrovascular lesions and genetic variants related to Hcy metabolism, as secondary outcomes, in the cognitive decline and dementia in Parkinson's disease (PD) were studied. This case-control study focused on 89 PD patients of minimum 10 years of evolution and older than 60 y.o., who were neuropsychologically classified either as cognitively normal (n= 37), having mild cognitive impairment (Petersen criteria) (n=22) or suffering from dementia (DSM-IV) (n=30), compared with cognitively normal age-matched control subjects (n=30). Plasma levels of Hcy, Vitamins B12 and B6, folic acid, polymorphisms in genes related to Hcy metabolism (*MTHFR*, *MTR*, *MTRR*, *CBS*) and silent cerebrovascular events were analysed. Plasma levels of Hcy were increased in PD patients (p=0.0001). There were no differences between the groups of patients. The brain vascular burden was similar among PD groups. There was no association between polymorphisms in the studied genes and the Hcy plasma levels or cognitive status in PD patients. We found no evidence for a direct relationship between Hcy plasma levels and cognitive impairment and dementia in PD. No indirect effect through cerebrovascular disease or genetic background was either found.

**Key words:** Homocysteine, Parkinson's disease, dementia, mild cognitive impairment, genetics, white matter hyperintensities

## INTRODUCTION

Dementia is a frequent manifestations of advanced Parkinson's disease (PD)<sup>1,2</sup>. Advanced age, longer duration of disease and greater severity are associated with dementia in PD<sup>1</sup>. Elevated plasma homocysteine (Hcy) levels represent a risk factor for cognitive decline and dementia in the general population<sup>3</sup> and have been associated with mild cognitive impairment (MCI)<sup>4</sup>, Alzheimer's disease (AD)<sup>4,5</sup>, vascular dementia<sup>6</sup> and depression<sup>7</sup>. In patients with PD, levodopa treatment leads to increased Hcy plasma levels,<sup>8-11</sup>. Hcy is regulated by several metabolic pathways (Figure 1) and may be determined by genetic and dietary factors. Genetic polymorphisms in genes encoding enzymes involved in Hcy metabolism could be partly responsible for the hyperhomocysteinemia observed in some PD patients. For instance, remethylation of Hcy may be more or less efficient depending on the activity of the enzyme *MTHFR* (5,10-Methylen-tetrahydrofolate reductase), which is determined by different genotypes<sup>12</sup>. The T/T variant of the genotype C677T of the *MTHFR* reduces its enzymatic activity, leading to higher levels of Hcy in patients treated with levodopa<sup>12-14</sup>. However, this association has not been found in other studies<sup>15</sup>. All of the above leads to postulate a role for increased Hcy levels as a risk factor in the development of dementia in PD. Indeed, several open trials have been performed to reduce Hcy levels in PD patients using vitamin B12, folate supplements or treatment with catechol-o-methyl transferase (COMT) inhibitors<sup>16-20</sup>. This leads to the somewhat irregular situation where a therapeutic approach is tested for an association (i.e. high Hcy levels and dementia in PD) which has not been proven<sup>21</sup>. Current information is limited to a few studies<sup>10, 22-24</sup> which have provided contradictory results. We analyzed a large group of PD patients to

extensively assess the possible correlation between Hcy plasma levels and the development of dementia.

## **METHODS**

The Ethics Committee for Medical Research approved the study. All patients and controls provided written informed consent. For patients with dementia, this was obtained from a relative. PD patients diagnosed according to the UK Brain Bank criteria<sup>25</sup> were recruited from our movement disorders clinic. Patients were older than 60 y.o. and had disease duration of 10 years or longer. Patients with other brain disorders, vascular or severe systemic disease, major psychiatric illness, previous cerebral surgery, or receiving anticonvulsants, vitamin B, folate supplements or COMT inhibitors were excluded. The Hoehn and Yahr scale and the Unified Parkinson's Disease Rating Scale motor section (UPDRS-III) were used to evaluate disease severity. Patients were classified as being cognitively normal (PD-CN), with mild-cognitive impairment (PD-MCI) and demented (PD-D). MCI was defined according to Petersen et al's criteria<sup>26</sup> which includes memory complaint, normal activities of daily living, normal general cognitive function, abnormal memory for age, and absence of dementia. The DSM-IV criteria were applied for diagnosis dementia. Patients exhibited multiple cognitive deficits (table 2) and impairment of daily life activities. Healthy controls were recruited among members of the Association of Blood Donors of Navarra (Spain). Cases with history of any neurological, psychiatric or major medical illness, with scores below normal in the neuropsychological assessment or with abnormalities in the MRI were ruled out.

The primary aim of this study was to determine the relationship between Hcy plasma levels and cognitive decline in advanced PD patients. Secondary objectives were the role of genetic factors related to Hcy metabolism and of silent vascular events in the cognitive decline of PD patients and their possible association with Hcy levels. The relationship between depression and Hcy and the role of factors such as vitamins Bs and folate plasma levels were also investigated.

#### *Neuropsychological evaluations*

Global cognitive function was evaluated with the Mini Mental State of Folstein (MMSE)<sup>27</sup> and the Blessed cognitive scale (BDS)<sup>28</sup>. Activities of daily living were evaluated with the Interview for Deterioration in Daily Living in Dementia scale (IDDD)<sup>29</sup>. Depression was rated using the Yesavage Geriatric Depression Rating Scale (GDS)<sup>30</sup>. The different cognitive domains were evaluated by the following tests: verbal episodic memory (Free and Cue Selective Reminding test of Buschke<sup>31</sup> and Cerad word list<sup>32</sup>); visual episodic memory (copy and delayed recall of two simple figures (Massachusetts General Hospital, Boston)); language (Boston naming test)<sup>33</sup>; attention and executive functions (Raven Progressive matrices<sup>34</sup>, semantic (animals) and phonetic (words starting with “p”) verbal fluency<sup>35</sup>, Trail Making Test parts A and B<sup>36</sup>, the Stroop test and digit span forward and backwards<sup>37</sup>). All tests were blindly applied by the same person in each center to control subjects and patients while under treatment, and were used alongside the diagnostic criteria described above to determine the cognitive status.

#### *Biochemistry*

All blood samples were obtained by antecubital venopuncture under fasting conditions and 4-6 hours after the first morning levodopa dose in PD patients. Plasma levels of Hcy, Vitamin B12 and folate were determined using a solid phase competitive

chemiluminescent enzyme immunoassay (INMUNOLITE 2500). Vitamin B6 was determined by radioisotopic assay (BUHLMANN laboratories Ag, Switzerland ALLSCHWIL).

#### *Genetic study*

Seven polymorphisms in genes involved in Hcy metabolism were typed using either restriction fragment length polymorphism (RFLP) assays or allele specific amplification with the primers and conditions available upon request. The polymorphisms included in this study were rs1801133 (c.C677T, p.A222V) and rs1801131 (c.A1298C, p.E429A) in *MTHFR* (accession numbers: NM\_005957, NP\_005948), rs1805087 (c.A2756G, p.D919G) in methionine synthase (*MTR*) (accession numbers: NM\_000254, NP\_000245), rs1801394 (c.A66G, p.I22M) in methionine synthase reductase (*MTRR*) (accession numbers: NM\_002454, NP\_002445) and c.844ins68, rs5742905 (c.T833C, p.I278T) and rs11700812 (c.G919A, p.R369H) in Cystathionine  $\beta$ -synthase (*CBS*) (accession numbers: NM\_000071, NP\_000062).

#### *Magnetic Resonance Imaging (MRI)*

All patients had an image study to exclude other causes of cognitive impairment. Because of practical reasons, in 18 cases only a CT brain scan could be performed. A 3-D structural MRI (1.5 T Magnetom SP (Siemens Erlangen, Gr) was performed in the rest of patients and controls using a T1-weighted MPRAGE sequence, (TR/TE/TI/NEX 1900/3.93/1100/1, flip angle 15°), matrix size 256 × 192, yielding 144 coronal slices and a slice thickness of 1.5 mm with in-plane resolution of 0.9765625 × 0.9765625 mm. FLAIR and T2-weighted sequences were also obtained. All MRI studies were undertaken

the same week that the neuropsychological and physical evaluation by the same radiologist who was not aware of the cognitive status of the patients and the Hcy levels. Brain vascular burden (white matter hyperintensities-WMH) was analyzed on FLAIR and T2 weighted MRI sequences. WMH were defined as hyperintense lesions on both sequences images. Only deep lesions of more than 5mm were counted. Caps and periventricular lesions less than 10 mm thick were not taken into consideration. WMH were semi-quantified using the ARWMC (age related WMH) scale of the European Task Force for the study of leukoaraiosis (Wahlund scale) 38. This scale was blindly applied by 2 neurologists.

### *Statistics*

The normal distribution of all variables was assessed using the Kolmogorov-Smirnov test. Distribution of the variables among the different groups (table 1) was assessed using Kruskal Wallis, ANOVA and mid-p test depending on the distribution and characteristics of the variable. A logarithmic transformation was applied as needed for the regression models. For the primary end point, a multinomial logistic regression was used for analysis. Secondary end points were assessed by linear multiple regression. Post-hoc Bonferroni's correction for multiple comparisons was applied. The statistics package SPSS 15.0 (SPSS Inc. Chicago, Illinois) and WinPepi 1.66 (PEPI-for-Windows) was used.

## **RESULTS**

Eighty-nine patients with PD classified as PD-CN (n=37), PD-MCI (n= 22) and PD-D (n=30) and 30 controls were studied.



The general characteristics of the groups are shown in table 1. PD-D patients were older than all other groups and had a higher depression score (table 1). Patient groups were comparable regarding years of evolution, severity of the disease and daily levodopa dose. All patients were receiving treatment with levodopa and 53 received additional treatment with a dopamine agonist (25 pramipexol, 14 ropinirol, 10 cabergoline, 4 pergolide). Sex distribution and level of education were similar among groups.

#### *Hcy plasma levels and biochemical data*

A linear multiple regression model for possible variables influencing Hcy levels was undertaken. Sex, disease duration and levodopa daily dose were not associated with Hcy plasma levels. Age, was positively associated with Hcy plasma levels. Acid folic, Vit B12 and Vit B6 were inversely associated with the plasma concentration of Hcy (global model:  $p=0.00015$ , adjusted R square =0.305). Plasma levels of vitamins B12 and B6 and folate did not differ among patient groups.

Plasmatic Hcy levels were higher (table 1) in PD patients than in controls ( $p= 0.0001$ ). Hcy levels did not predict cognitive status ( $p=0.403$ ) in a multinomial logistic regression of PD patients adjusting for variables influencing Hcy plasma levels and cognitive decline (age, Vit B12, Vit B6 and folic acid). Results were similar when pooling together PD patients with any degree of cognitive impairment (i.e. PD-MCI plus PD-D). No correlation was found between Hcy plasma levels and performance on neuropsychological tests (table 2).

#### *Genetic Study*

The control population was in Hardy-Weinberg equilibrium for all polymorphisms analyzed except for the *MTRR*-A66G. For this reason, this SNP was excluded from the

analysis. All three polymorphisms in the *CBS* gene were in complete linkage

disequilibrium, and therefore only data for the c.844ins68 polymorphism is shown.

Due to the low number of individuals homozygous for the mutant genotype in some polymorphisms, the analysis was performed grouping together those genotypes carrying at least one mutant, i.e. less frequent allele (TT plus CT for the c.C677T *MTHFR*; GG plus AG for the *MTR* c.A2756G; ins+/ins+ plus ins+/ins- for the c.844ins68 *CBS*).

Linear multiple regression analysis for variables influencing Hcy concentration (age, Vit B12, Vit B6 and folic acid) was applied to assess whether or not these polymorphisms influenced Hcy plasma levels. This revealed that Hcy levels are independent of the genetic variants (table 3). Moreover, we found no relation between the genetic variants and Hcy plasma levels in the control group.

A multinomial logistic regression model adjusting for age was applied to assess the effect of the genetic variants in the cognitive status of PD patients. There was no association between any polymorphisms and the cognitive status even when pooling together all patients with some degree of cognitive impairment (i.e PD-MCI plus PDD patients) (table 4). None of the studied polymorphisms was associated with increased risk of PD.

#### *White matter changes in MRI*

In 3 patients the Flair sequences of the MRI were not suitable to evaluate the Whalund scale, so sixty-eight patients were available for analysis. The scores on the Wahlund scale were  $1.32 \pm 1.96$  in PD-CN;  $1.57 \pm 2.14$  in PD-MCI and  $2.10 \pm 2.19$  in PD-D patients.

Adjusting for age, there were no differences among the groups of patients ( $p=0.958$ ).

Interestingly, age and Hcy plasmatic levels were independently associated with the Wahlund scores, but there was no association when both variables were included in a

multinomial logistical regression model. This could be due to the fact that silent vascular events and Hcy plasmatic levels increase with age.

### *Depression*

Patients with PD-D ( $p < 0.001$ ) and with PD-MCI ( $p = 0.007$ ) were more depressed than PD-CN patients (GDS=  $11.81 \pm 4.30$  in PD-D;  $10.60 \pm 4.9$  in PD-MCI and  $5.85 \pm 3.70$  in PD-CN). There was no difference between PD-CN patients and controls (GDS=  $4.5 \pm 3.7$ ). No correlation between Hcy plasma levels and depression ( $r = 0.154$ ,  $p = 0.233$ ) was found. None of the variants of the polymorphisms studied was associated with depression.

## **DISCUSSION**

### *Hcy plasma levels and dementia*

We found no relationship between Hcy plasma levels and cognitive impairment in patients with PD. Our pilot study included an extensive analysis of a fairly large cohort of PD patients and a detailed neuropsychological evaluation that allowed appropriate classification according to the cognitive status. After adjusting for factors influencing Hcy plasma levels and cognitive decline in a multinomial logistic regression model, we found that Hcy plasma levels did not predict the cognitive status of PD patients.

Although a ceiling effect in all PD groups due to the effect of levodopa on Hcy may be partially responsible for this negative finding, we failed to detect any association between levels of Hcy and cognitive status or neuropsychological performance. Previous studies investigating this subject yielded contradictory results. Higher Hcy plasma levels in patients with cognitive decline than in non-demented patients<sup>39</sup> were reported in a

retrospective study conducted in a reduced number of patients in whom the MMSE was the only test used for evaluation. Religa et al<sup>10</sup> found a correlation between high Hcy plasma levels and low MMSE scores. However, the PD group had low vitamin B12 levels, which might be a confounding factor affecting these results. High levels of Hcy have also been associated with poorer performance in frontal and memory tests<sup>22</sup> in PD. On the other hand, one prospective study in early PD patients showed no difference in motor, mood and cognitive deterioration between patients with and without high Hcy levels after levodopa treatment was initiated,<sup>23</sup> suggesting that disease progression is not associated with Hcy plasma concentration. Similar negative findings were reported in a cross-sectional study of 72 PD patients in whom there was no association between Hcy and depression, cognitive performance and psychosis<sup>24</sup>. A recent study of demented PD patients showed a similar degree of cognitive impairment in patients with low and high Hcy levels<sup>40</sup>. In our study, patients were evaluated thoroughly from a neuropsychological point of view and the patient population was sufficiently old and had suffered PD for long enough to be at risk of developing dementia<sup>1</sup>. This makes it very unlikely that our negative findings could be explained by a population bias. Moreover, our statistical analysis was corrected for age and disease duration, and no significant correlation was found. We also took care to differentiate PD patients with MCI and dementia under the hypothesis that MCI could be the earliest phase of cognitive decline in PD<sup>41</sup>, so our patient population was more accurately classified than in previous studies. While the appropriateness of Petersen's criteria to evaluate MCI in PD may be questioned<sup>42</sup>, the fact is that by analyzing (separately or together) PD patients with dementia and with MCI, we should have increased the sensitivity to detect any possible

association between plasma Hcy levels and cognitive impairment. Moreover, we found no correlation between the scores on the neuropsychological tests and Hcy plasma levels. We also assessed the role of ischemia as a factor associated with dementia.

Hyperhomocysteinemia is a risk factor for ischemia and vascular dementia<sup>6</sup>, and there is an association between WMH and cognitive impairment and dementia<sup>43</sup>. The incidence of WMH in all groups of patients was not statistically different. We excluded from the study patients with severe vascular risk factors and recognized vascular diseases, but the possibility of mild vascular pathology can not be entirely ruled out.

#### *Hcy metabolism, genes and dementia*

Hcy plasma levels may also be influenced by genetic factors that determine a less efficient metabolism of Hcy. These include the following (figure 1): 1.excessive production of S-adenosyl homocysteine when levodopa is metabolized by COMT; 2. reduced remethylation of Hcy due to exhaustion of the pool of methyl donors, as in folate and/or vitamin B12 deficiency states or in individuals with genetically determined inefficiency of MTR or MTHFR; 3.deficient transulphuration pathway which can be genetically determined or result from vitamin B6 deficiency. Thus, hyperhomocysteinemia can arise from the effect of several polymorphisms on genes involved in Hcy metabolism. In the general population, possession of the T allele in *MTHFR* c.C677T, the C allele in *MTHFR* c.A1298C, the A allele in *MTR* c.A2756G or the c.844ins68 allele in *CBS* all caused hyperhomocysteinemia. However, only the *MTHFR* c.C667T polymorphism has been studied in PD patients. The TT variant of this polymorphism has been associated with higher Hcy plasma levels in PD patients treated with levodopa<sup>12-14, 44, 45</sup>. Other studies have shown that this variant does not influence Hcy plasma levels in levodopa-treated PD patients<sup>10</sup> or in patients not receiving

levodopa<sup>13</sup>. Considering a possible interaction between genetic variants and levodopa intake in Hcy levels, we performed an extensive genetic assessment in this study. Plasma levels of Hcy were independent of genetic variations for every one of the polymorphisms and no association was found between the different polymorphisms studied and the cognitive status in PD patients. Admittedly, it could be argued that the influence of levodopa on Hcy levels is so overt that it makes it unlikely that any influence of these polymorphisms could be observed.

In conclusion, we studied a patient population representative of advanced PD, and failed to find any evidence indicating that high Hcy plasma levels are a risk factor for cognitive decline. No association between Hcy plasma levels or polymorphisms of genes implicated in its metabolisms and cognitive impairment or dementia was found in PD patients. However, the putative role of high Hcy levels in PD dementia cannot be entirely discarded given the pivotal role of Hcy in cellular metabolism. A prospective, large scale study with unselected PD patients is required for definitive ascertainment.

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## **Author Roles**

Rodriguez-Oroz MC. Conception, general organization, coordination and supervision of the study, statistical analysis and interpretation of the results and writing of the article, as well as selection and motor evaluation of patients. The principal author MCR had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Pérez-Tur J. Genetic study, statistical analysis of data and interpretation of results. Review of the manuscript.

Obeso JA. Conception of the study, selection and motor evaluation of patients. Critical discussion of draft.



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**Tables and figure legends:**

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Figure 1. Diagram showing the effect of levodopa on the metabolism of homocysteine. Note: the reaction involving BHMT is absent in from the brain.

**Table 1 General characteristics of the patients and controls and plasma levels of homocysteine and cofactors in its metabolism**

	Control(n=30)	PD-CN (n=37)	PD-MCI(n=22)	PD-D(n=30)
Age	68.48 ± 2.98	69.97 ± 6.5	70.23 ± 5.2	74.87 ± 6.15**
Sex ( % male)	51.72	54.55	63.64	60.00
Years Evol	-----	14.68 ±4.62	13.05 ± 3.69	14.73 ± 4.45
Educ ( %Pri)	68.97	65.00	91.67	81.25
UPDRS OFF	-----	41.88 ±9.54	43.75 ± 4.76	48.11 ± 7.16
Hoehn and Yahr	-----	3.35 ± 0 .91	3.43 ± 0 .62	3.94 ± 0.52
Depression (GDS)	4.4 ± 3.3	6.53 ± 5.02	9.41 ± 5.17 <sup>0</sup>	12.13 ± 4.73 *
Ldopa(mg/day)	-----	786.11 ± 262.68	825.04± 331.16	811.32± 338.09
DA agonists (%)		66.66	63.63	56.66 <sup>&amp;</sup>
Hcy (µmol/l)	8.55 ± 1.95***	14.9 ± 4.7	15.1 ±4.3	15.4 ±5.4
Vit B12 (pg/ml)	-----	400.1± 222.2	353.2 ±205.6	380.2 ±156.5
Vit B6 (nmol/l)	-----	49.5 ± 31.6	65.7 ±56.0	49.7 ±63.3
Folate (ng/ml)	-----	10.2 ± 5.6	11.6 ±6.5	10.4 ±4.6

Educ ( %Pri): Educational level ( % of primary school)

DA agonist (%) Percentage of patients taking dopamine agonists

**\*\* P <0.0001 vs control and PD-CN; p=0.02 vs PD-MCI**

**\* P <0.001 vs PD-CN**

**<sup>θ</sup>p= 0.04 vs PD-CN**

**<sup>&</sup>p= 0.04 vs PD-CN**

**\*\*\* P =0.0001 between groups**

**Table 2. Summary of the scores of the neuropsychological tests in every group and correlation of the scores in the whole population of PD patients with Hcy plasma levels.**

Test	Cut-off scores	Controls	PD -CN	PD-MCI	PD-D	Pearson Correlation coefficient (r) in PD patients
MMSE*	≤24	28.93 (1.28)	29.0 (2.25)	25.0 (5.50)	19.0 (5.0)	-0.176
IDDD	>35	33	35 (2.31)	35.4(3.2)	54.8 (14)	
Digit span	≤10	10.32 (2.11)	12.57 (3.27)	8.38 (3.58)	6.14 (3.08)	-0.056
Figure copy*	≤8	10. 0 (0.0)	10.0 (0.0)	10.0 (0.0)	8.0 (4.0)	0.032
Figure recall*	≤6	6.48 (4.0)	8.0 (4.0)	2.0 (10.0)	0.0 (0.5)	0.051
Phonemic fluency	≤10	13.86 (5.60)	13.79 (6.34)	10.13 (6.47)	7.43 (3.55)	-0.060
Semantic fluency	≤15	18.07 (4.83)	18.64 (4.75)	10.50 (2.72)	8.0 (3.11)	-0.021
FCSRT*	≤ 44	47.41 (0.8)	48.0 (1.0)	47.0 (7.75)	28.29 (24)	-0.136
Boston*	≤49	49.21 (5.52)	53.50 (6.25)	40.5 (14.25)	30.0 (5)	0.015
Raven	≤26	26.93 (3.67)	26.0 (3.68)	17.75 (6.25)	13.57 (3.73)	0.038
Trail A	≥70 sec (60-69 y.o) ≥100 sec (70-79 y.o)	65.69 (29.42)	56.0 (24.10)	115.75(47.36)	233.58 (77.35)	0.114
Trail B*	≥170 sec (60-69 y.o) ≥280 sec (70-79 y.o)	137.31 (55.63)	140.0 (145)	245.0 (125.0)	301.0 (0.50)	-0.064
Stroop words	≤40	48.37 (6.27)	40.79 (11.01)	31.0 (8.70)	29.29 (6.16)	-0.102
Stroop color-word	≤40	43.93 (6.03)	43.78 (8.65)	37.92 (6.68)	28.56 (13.5)	-0.154

Mean and standard deviation, except tests with \*, where median and interquartile range is

shown. FCSRT (Free and cued selective reminding test).

**Table 3. Plasmatic levels of homocysteine corresponding to the different variants of the genetic polymorphisms in PD patients.**

GENE	VARIANT	Hcy	*p-value
MTHFRC677T	CC (n=33)	15.75 ± 4.19	reference
	CT (n=41)	14.45 ± 5.50	0.255
	TT (n=3)	13.76 ± 3.80	
MTHFRA1298C	AA (n=33)	15.07 ± 4.69	reference
	AC (n=39)	14.59 ± 5.32	0.336
	CC (n=10)	16.06 ± 3.47	0.181
MTRA2756G	AA (n=59)	15.53 ± 5.01	reference
	AG (n=21)	13.79 ± 4.27	0.152
	GG (n=2)	10.49 ± 0.55	
CBS 844ins68	I/I (n=68)	14.84 ± 4.95	reference
	I/D (n=11)	14.19 ± 3.12	0.299
	D/D (n=3)	20.44 ± 5.76	

\*p value for the polymorphism included in the multiple regression model adjusted for age, Vit B12, Vit B6 and folic acid.



**Table 4. Genotype distribution at the polymorphisms analyzed stratified by population.**

Gene	cDNA (Protein)	Control group			Whole PD group (%)			Cognitive normal PD (%)			PD-MCI (%)			PD-D(%)		
		C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T
MTHFR	C677T	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T	C/C	C/T	T/T
	(A222V)	9	13	6	33	41	3	19	12	3	5	16	0	9	13	0
		(32.1)	(46.5)	(21.4)	(42.9)	(53.2)	(3.9)	(55.9)	(35.3)	(8.8)	(23.8)	(76.2)	(0.0)	(40.9)	(59.1)	(0.0)
	p						0.0239*									0.080&
	A1298C	A/A	A/C	C/C	A/A	A/C	C/C	A/A	A/C	C/C	A/A	A/C	C/C	A/A	A/C	C/C
	(E429A)	15	9	2	33	39	10	13	19	4	10	10	2	10	10	4
		(57.7)	(34.6)	(7.7)	(40.2)	(47.6)	(12.2)	(36.1)	(52.8)	(11.1)	(45.5)	(45.5)	(9.0)	(41.7)	(41.7)	(16.6)
	p						0.3277*									0.901&
MTR	A2756G	A/A	A/G	G/G	A/A	A/G	G/G	A/A	A/G	G/G	A/A	A/G	G/G	A/A	A/G	G/G
	(D919G)	19	9	1	59	21	2	20	14	2	20	2	0	19	5	0

		(65.6)	(31.0)	(3.4)	(72.0)	(25.6)	(2.4)	(55.6)	(38.9)	(5.6)	(90.9)	(9.1)	(0.0)	(79.2)	(20.8)	(0.0)
	p	0.6580*						0.021&								
CBS	844ins68	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D
		26	3	0	68	11	3	33	2	1	18	3	1	17	6	1
		(89.7)	(10.3)	(0.0)	(82.9)	(13.4)	(91.6)	(5.6)	(2.8)	(4.5)	(81.8)	(13.6)	(4.5)	(70.8)	(25.0)	(4.2)
	p	0.6437*						0.141&								

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\*: P-values obtained comparing the genotype distribution at each SNP between cases and controls. All analysis was performed with 2 degrees of freedom. Corrected  $\alpha$  value due to multiple comparisons: 0.0125

& p-values obtained comparing the different groups of PD patients. Likelihood-ratio test  $p$ -value when the genetic polymorphism is added to the model adjusted for age. Corrected  $\alpha$  value due to multiple comparisons: 0.0125

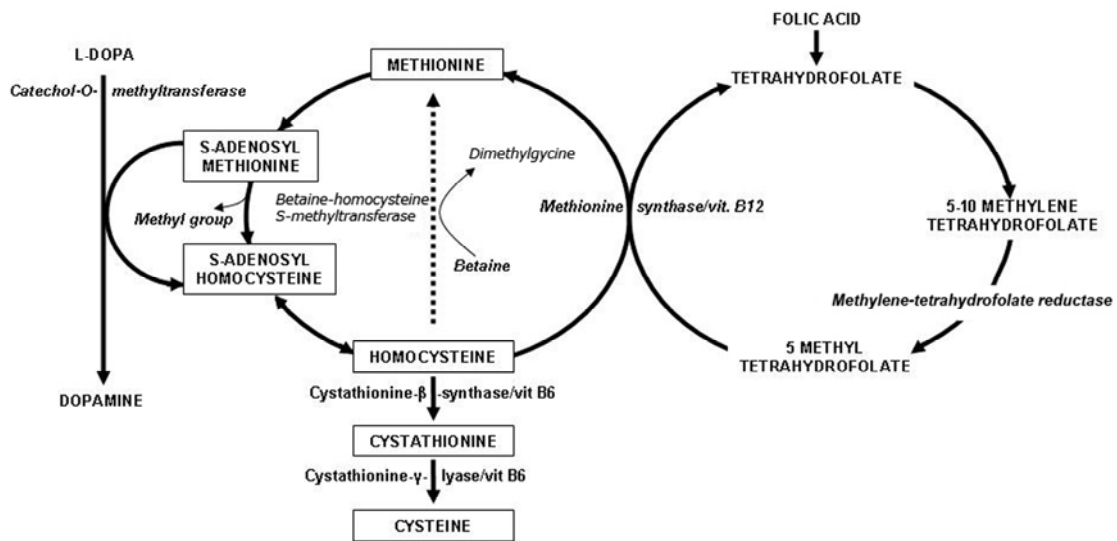


Figure 1. Diagram showing the effect of levodopa on the metabolism of homocysteine.

Note: the reaction involving BHMT is absent in from the brain